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GENERAL BIOLOGY OF THE PROTOZOAN LIFE CYCLE[1]

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FOR five decades after the time of Ehrenberg, the peculiar conception of a protozoan as a miniature replica of a metazoan, held by this gifted observer, influenced the study of Protozoa. This influence gradually wore off and, so far as morphology is concerned, ended with the careful observations of Stein, Claparède and Lachmann, Engelmann, Bütschli and Hertwig, who showed that various structures of the protozoan body are not beating hearts, brains, ovaries and stomachs, but are simple differentiations of the single-celled organisms.

A more lasting influence of Ehrenberg's teaching, seen even to-day, is the habit of regarding a single protozoon as the complete expression of a species equivalent to an individual worm, mollusc or mammal. The individual metazoon dies, while the protozoon does not die but grows to full size and divides into two or more—facts which led Weismann to his conclusions regarding mortality in Metazoa and immortality in Protozoa.

We owe to Maupas the credit for dissipating this last reminiscence of Ehrenberg's teaching, and for showing that the single cell is not the final representative of a protozoon species. We are accustomed to the idea that many

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individuals of a polymorphic coelenterate are present in potential in the fertilized egg of the coelenterate, but we are less accustomed to the idea that polymorphic individuals are present in potential, in the fertilized cell of a protozoon. Research in recent years has shown that successive generations of Protozoa may be more or less progressively differentiated, so that a cell picked out at one phase of the life cycle is quite a different type of individual from one picked out at another phase. Which, for example, would be the "type" individual of the dimorphic Foraminifera? Which would be the type in the reproducing flagellated and ameboid stages of *Nägleria punctata?* of different phases in the life history of *Centropyxis, Arcella,* or *Difflugia?* or of intestinal and blood-dwelling stages of *Plasmodium?* The morphological differences here indicate that the protozoan life history involves differentiation analogous to that of a polymorphic metazoon, and justify the comparison of the whole life cycle with the development and differentiation of a metazoon, especially that of a metagenetic type such as coelenterate or trematode.

The importance of the whole life cycle, first demonstrated by Maupas, was fully recognized by Schaudinn and applied by him to the study of parasitic forms. The monographs resulting from this study, especially those on *Coccidium schubergi, Plasmodium vivax* and on rhizopods, are classics in the literature of Protozoa, and models which later students have followed.

Through Schaudinn's work, and by later researches, the sequence of events in different parasitic types has been made out with painstaking care until to-day, we know the general history of the majority of injurious human protozoan parasites, the modes of transmission from host to host, the types of intermediate hosts and what happens in them. In short, we know enough to furnish an adequate basis for public and private prophylaxis which, in the hands of sanitary commissioners and public health officers, has put an end to epidemics of yel-

low fever, malaria and dysentery; has rehabilitated vast tracts of land in Italy; saved millions of dollars in South Africa and in our southern states, and has made the Panama Canal possible.

Such are the first, and practically the most important, results of our knowledge concerning protozoan life cycles; quite enough, indeed, to justify the science of Protozoology. Important as these results are, we are not at all satisfied; we know too little about the conditions of development; too little about the nature of the vital processes of the organisms themselves and their variations in structure and function under differing conditions, ignorance which must be cleared away before much further practical advance can be made. Further advance will be less spectacular and must be based upon the biological study of the organisms as units of protoplasmic substance, and this will rest upon working hypotheses supported by experiment. It is along such theoretical lines that I wish to direct your attention for a few minutes, to develop a conception of the life cycle as a whole, and to offer a theoretical interpretation of the different phases of vitality and of structural variations.

Let us consider for a moment, a single *Ameba* or a malaria germ, not as a cause of disease, but as a unit mass of protoplasm which, like a free-living *Paramecium* or *Didinium,* performs all of the fundamental vital activities common to living things, namely nutrition, excretion, irritability and reproduction. The chemical composition of these unit masses, so far as I know, has never been made out, but there is no reason to doubt that it agrees with that of other living substances, since the accompanying properties of protoplasm—metabolism, growth and reproduction—are obviously performed, and probably in the same way. In such unit masses of protoplasm we assume that processes of hydrolysis, synthesis, oxidation and reduction, are constantly going on as in other protoplasms, and not in any haphazard way, but always orderly and under regulative control of the organism as a whole.

The appearance of *Ameba* shows that the protoplasm is made up of alveoli and inter-alveolar substances of different density, representing colloidal and crystalloidal substances in a general mixture which Ostwald describes as an emulsoid. Between these different substances constant chemical activities are in progress, and the orderliness which distinguishes these processes in the protoplasm of the living organism from similar processes which go on in the same protoplasm when crushed, are possibly due, as Mathews states, to the physical barriers of cellular and nuclear membranes, alveoli, and the colloidal centers of activity. The speed with which such processes take place in living protoplasm, which, in itself, distinguishes living processes from chemical processes in lifeless substances, is due to specific enzymes or catalyzers which are manufactured as a result of chemical activities in living protoplasm. These bring about and control each successive step in the long chain of chemical actions involved in destructive metabolism, the action in each event being conditioned by the nature of the protoplasmic substratum. In this chain of destructive processes different substances may be formed which undergo no further oxidation or other chemical change, but are stored up in the protoplasm until disposed of by excretion, these products, leading to changes in the protoplasmic substratum, *i. e.,* to protoplasmic chemical differentiation, may or may not be accompanied by visible structural differentiations. Such products of destructive metabolism, in the form, usually, of nucleo-proteins or their derivatives, may act as poisons to other organisms, as melanin does to the host in malaria, or as the proteolytic ferments of *Entameba histolytica* do in dysentery; or they may play some important part in the vital activities of the organism itself, as in phosphorescence of *Noctiluca* and the dinoflagellates, or more generally, in regeneration and reproduction.

Let me illustrate this latter point by some experiments made on *Uronychia transfuga,* a ciliated protozoon. This organism has rather a complicated structure with nine

giant cirri at the posterior end (Fig. 1). Under laboratory conditions it divides once a day approximately, or, more exactly, once in twenty-six hours. The first indication of division is the precocious formation of the giant cirri in a central region of the body which we have called the "division zone." The experiments were undertaken for the purpose of studying the relative power of regeneration of the single cell at different ages between divisions, it having first been determined that the cell regenerates readily after being cut. Cells were cut with a scalpel at different periods subsequent to division; some during the end stages of division; some 15 minutes after division; some one hour after; others 2, 4, 8, 12, 16 and 20 hours after, and some were cut just prior to the next division period, *i. e.*, 24 to 25 hours after division. In all cases of record, the cells were so cut that one portion contained the micronucleus and part of the macronucleus, the other portion containing only a part of the macronucleus. The former, or, as I shall call it, the nucleated portion, invariably regenerated after some hours, forming a perfect cell, the latter, without a micronucleus which I shall call the enucleated portion, behaved differently as regards regeneration, according to the age of the cell when cut. In all cases this portion lived from three to five days after the operation. If the recently divided cell were cut at any period up to 16 hours after division the result was the same; no regeneration occurred, the fragment merely rounded out, swimming about by its adoral membranelles (Fig. 2, 3). If the cells were cut when from 18 to 24 hours old, regeneration occurred not only in the nucleated portion, but in the *enucleated fragment as well,* the percentage of regeneration increasing with the increased age of the cells when cut, until at the age of 24–25 hours the enucleated fragments regenerated perfectly in 100 per cent. of cases (Fig. 4, 5, 6, 7).

These results indicate a gradual chemical differentiation of the protoplasm as a result, probably, of destructive and constructive metabolic processes. The giant

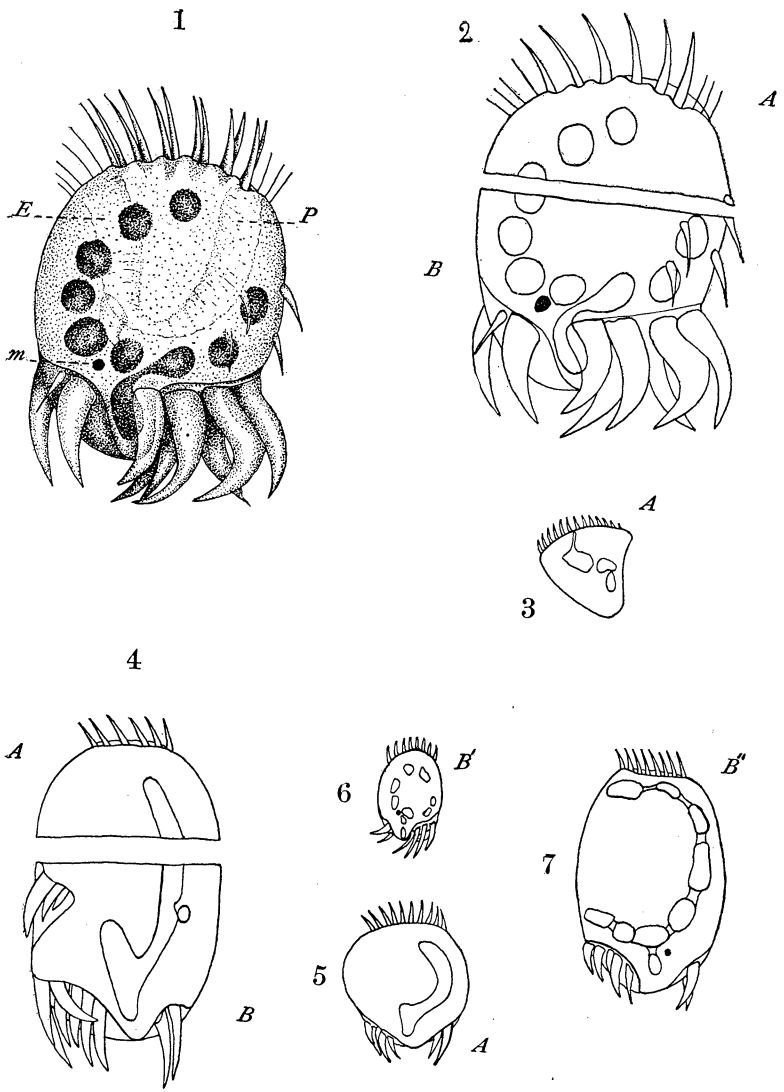

FIG. 1. Normal adult individual of *Uronychia transfuga* with macronucleus in the form of large chromatin spherules; micronucleus (*m*); endoral membrane (*E*); pre-oral membrane (*P*); and large posterior cirri.

FIGS. 2 AND 3. Individual 12 hours old cut as shown in 2. Part *A* had no micronucleus and after 72 hours appeared as shown in 3 *A*. Part *B* regenerated perfectly in 24 hours.

FIGS. 4, 5, 6 AND 7. Individual cut at age of 25 hours as shown in 4. *A* regenerated perfectly, except for absence of micronucleus, in 24 hours (5 *A*); *B* divided through the original division plane (indicated in 4), within a few hours forming a minute but perfect individual (6B'), and a normal full-size individual (7 *B''*).

cirri which are regenerated are the visible expression of inherited structures characteristic of the species. Since the enucleated fragment from a cell cut when young does not regenerate while the nucleated fragment does, we must conclude that one essential factor at least, necessary for the production of these inherited structures, lies in the micronucleus.

The giant cirri, furthermore, are visible differentiations which are precociously formed at division. This must mean that the inherited factors find their expression at this period, and it follows from the successful formation of giant cirri in enucleate fragments from old cells, that whatever may be the direct causative agent or agents in the process they must be generally distributed throughout the protoplasm at this time. We have no direct evidence as to what these agents may be; possibly there is only one and that of the nature of a specific enzyme, or perhaps some chemical body analogous to hormones formed as a result of mutual interaction of nucleus and cytoplasm when the latter has reached a certain stage of chemical differentiation through normal activities. Or it is possible that such chemical bodies are present at all times and are activated only when the protoplasmic substratum reaches some particular stage of development. Thus it is possible that, with continued metabolism, the acidity of the protoplasm gradually increases until a concentration is reached in which specific enzymes, not able to act before, are now activated.

However theoretical the interpretation of the phenomenon may be, the periodic and temporary power of regeneration is an observed fact indicating a difference in the protoplasmic make-up at different age periods, a difference which may be satisfactorily expressed by the phrase cumulative chemical differentiation.

Another observed fact is that the regenerative power is exhausted with cell division, for young enucleated fragments do not regenerate. This indicates a reduction of the differentiated adult protoplasm to the condition of young cells; or, at least, the protoplasm is restored to a

state where the causes underlying regeneration are inactive. This may be due to the exhaustion of specific substances which take part in the reaction of regeneration, or it may be due to the chemical and physical changes accompanying cell division.

We are led through these experiments, to further speculations concerning the nature of cell division. Chemical differentiation of the protoplasm continues even after the stage is reached when regeneration is possible. This is shown by the fact that formation of the cirri in *Uronychia* precedes the process of division in normal cells, and by the additional fact that regeneration of cirri occurs while cell division does not occur in enucleate fragments cut from old cells. I would interpret cell division as due to cytolytic action set up by enzymes or other chemical bodies produced as a result of interaction of nucleus and cell body differentiated chemically by age. Cytolysis may then occur more or less extensively throughout the entire protoplasmic mass, but it is most active in the division zone of the organism which is more highly differentiated than other regions (see Calkins, 1911, and Peebles, 1912). The membrane of the cell turns in at this cytolyzed division zone and the constriction results in cell division.

As a consequence of the activities accompanying cell division the protoplasmic substratum is reduced from the differentiated adult condition to the condition characteristic of young cells, and the processes of growth and chemical differentiation, division and de-differentiation, recur in more or less rhythmical succession.

Viewing the life cycle as a whole, there are two phases which must be taken into account. These are, first, the encystment phase, and second, the sexual or conjugation phase, both widespread and almost universal in protozoan life histories. Let us first consider the encystment phase.

Encystment occurs ordinarily when the conditions in the surrounding medium are adverse, such as desiccation, lack of food, etc., such encysted forms emerging from the cyst when suitable conditions are restored. In some cases also, encystment occurs during the digestion of food. In

addition to these casual encystments there is another form of encystment which involves more deeply-lying activities of the protoplasm. In *Didinium nasutum* I have found that encystment occurs at periodic intervals which cannot in any way be connected with adverse conditions of the environment or with feeding, but must be interpreted as a normal phenomenon due to internal conditions of the organisms. Encystment at such times persists for from 5 to 8 days and during this period no amount of coaxing will bring the organisms out. During such encystment the macronucleus fragments into hundreds of small chromatin particles which are ultimately absorbed in the cytoplasm; the micronuclei divide, and products of their division give rise to a new macronucleus and new micronuclei. When the process is completed and the organisms emerge from their cysts they possess from five to seven times the vitality, as measured by the division rate, of the same race prior to encystment. Fermor was the first in 1913 to describe similar happenings during the encystment of *Stylonychia;* in this case, dissolution of the old macronucleus and absorption of the fragments, fusion of the two micronuclei and formation of new macronuclei and micronuclei from the fusion nucleus, were described.

It is well known that *Paramecium* does not encyst. Nevertheless Woodruff and Erdmann (1914) have shown that phenomena similar to those occurring during encystment in *Stylonychia* and *Didinium,* and which they refer to under the general term "endomixis," recur at periodic intervals (about once a month) in the case of *Paramecium aurelia.* Here also the old macronucleus fragments and the fragments are absorbed in the cytoplasm, while a new macronucleus and micronuclei are formed from the division products of the old micronuclei.

The interpretation of this set of phenomena in the life history of protozoa is a perplexing problem. There is not a doubt that vitality, as measured by the division rate, is restored. Likewise there is little reasonable doubt that a complete chemical and physical reorganization of the protoplasm takes place. The renewal of vitality was shown

both in Woodruff's culture and in my *Didinium* culture, and one general problem is stated in the query: how long can such periods of reorganization continue? Woodruff believes that they may keep on indefinitely, but in my experiments with *Didinium* the race apparently lost its power to encyst and ultimately died out after six months' culture without encystment. So too, in my culture of *Paramecium caudatum* (1902) where similar reorganization occurred at least twice, the race ultimately lost the power to reorganize and died out. I may have had unfavorable forms to start with and so lost both races at early dates. It is interesting in this connection, however, to note that Whitney, working with the rotifer *Hydatina,* a metazoon, carried a race through nearly 200 generations by parthenogenesis when the individuals lost their power to reproduce in this way, and many of his lines died, while others produced sexual individuals.

The general biological effect of this process of reorganization is a new chemical combination with a new potential of metabolic activity, and a new lease of life. Not only are the nuclei restored to activity, but the cytoplasm is likewise completely reorganized by the distribution through it of relatively large quantities of nucleo-proteins, giving rise to successive derivatives (through hydrolysis, oxidation, reduction, etc.), all increasing the metabolic processes and releasing more chemical energy expressed by activity of movement and feeding, and leading to more rapid assimilation and growth, all indicated by an increased division rate. In short, the protoplasm is rejuvenated.

The second phase in the life history to be considered, viz., the sexual phase, involves still more deeply-reaching protoplasmic activities. The protoplasm of the individual cells at this period has a different physical, and presumably chemical, make-up than during ordinary vegetative periods. In free-living forms, such as the ciliates, the outer protoplasm becomes sticky or glutinous so that two cells on touching, fuse together. In this condition which I have called the "miscible state" conjugation is possible, and the physical condition may be so extreme that groups

of cells get stuck together. I have witnessed the fusion of nine *Paramecium caudatum* cells in a single amorphous mass.

In other forms, notably the parasitic protozoa, protoplasmic changes at this stage follow two lines of differentiation. Some cells store up metabolic products in the form of reserves of nutriment and develop into female gametocytes or macrogametes. Others develop into more active male gametocytes and microgametes. In both of these differentiated types if union or fertilization is prevented, the cells die a natural death.

The effects of conjugation or fertilization are almost the same as those following asexual reorganization through encystment. In ciliates cytolysis of the old macronucleus takes place and its substances are absorbed, that is, undergo chemical changes in the cytoplasm. The majority of the maturation nuclei, both in free-living and in parasitic forms, meet the same fate, while a new nuclear apparatus results from the products of the fertilization nucleus or synkaryon. The cytoplasm is renewed in a chemical sense and metabolic activities recommence with renewed vigor; a new race is started. The sole difference from encystment is that reorganization occurs after or during amphimixis and a new hereditary complex is formed in the nucleus, while even this, in endogamous conjugation at least, can not be very different from the condition after asexual reorganization. It is obvious that, if conjugation is the equivalent of fertilization in metazoa, asexual reorganization or endomixis is the equivalent of parthenogenesis.

What is the significance of these two important phases in the life cycle and how can they be interpreted in terms of metabolic activities? As we have seen, there is reason to believe that the cell protoplasm becomes progressively differentiated in a chemical sense between division periods, until just prior to division processes take place which do not occur at earlier periods. With division this differentiated condition is reduced, possibly through cytolysis, until a more labile protoplasm results. Now it is not at

all improbable that such reducing processes are more or less incomplete, so that the protoplasmic substratum in the second generation is different from that of the first. We have evidence of this in the foraminifera where differences in the protoplasmic structure and in shell structure characterize the second generation. Further evidence is seen in the rhizopods, where increasing quantities of chromidia, and in some cases differences in shell structure, are morphological indications of differentiation.

Furthermore, it is not improbable that such differences are cumulative from generation to generation, just as chemical differentiation is cumulative with inter-divisional age, until a protoplasmic substratum is evolved in which processes not possible before can now take place. We have shown that *Paramecium* at the conjugation phase has a different physical make-up than at other times, the cortical plasm becomes mucilaginous and fusion results on contact, while physiological differences are manifested by the invariably decreasing division rate during and after this period when conjugation is possible. Here the protoplasmic substratum is differentiated, and processes occur which are not possible at other times. So, too, in *Didinium, Stylonychia,* etc., with successive generations a protoplasmic substratum is gradually evolved (possibly hastened by adverse conditions) in which the peripheral zone of protoplasm undergoes cytolysis and forms an impervious membrane—the cyst membrane—analogous to the fertilization membranes of metazoan eggs. Further cytolytic changes, involving hydrolysis, reduction and other chemical activities, are set up in the cell body, especially in the cell nuclei which divide or fragment. As a result of these activities, which are more profound than those accompanying cell division, the protoplasm is again restored to a labile condition, vitality is renewed and a de-differentiated protoplasm begins a new cycle of metabolic and reproductive phases.

The phenomena of conjugation may be interpreted in a similar way as due to processes possible only in a substratum produced by cumulative protoplasmic differentia-

tion. A visible expression of such differentiation is seen again in the chromidia formation of *Sarcodina* and in the dimorphic gametocytes of foraminifera and Sporozoa. The reorganization phenomena are quite as complicated and as far reaching as after encystment, and the end result is the same, a de-differentiated protoplasm and a new individual with a high potential of vitality. If fertilization is prevented the differentiated macro- and microgametes die as do metazoan eggs and spermatozoa, and a similar result follows the continued culture of free-living ciliates in which conjugation, or its equivalent, asexual endomixis, is prevented.

In all life histories we find more or less regular cycles of vegetative and sexual phases, complicated by more or less active asexual and sexual reproduction. In parasitic forms it is possible, I may say probable, that reorganization and renewal of vitality take place during encysted stages as Schaudinn, Wenyon and others have held for the genus *Entameba;* or, as in *Paramecium,* they may take place without encystment in types like *Plasmodium* as described by Schaudinn. The processes of autogamy, so-called, described for different types of *Entameba,* may be interpreted as asexual endomixis, and the conflicting views as to the significance of nuclear structures in *Entameba coli, E. histolytica, E. tetragena* and *E. minuta,* may all be reconciled when this possibility of asexual reorganization is applied to the various parasitic rhizopods.

With *Plasmodium,* the principle of asexual reorganization and renewal of vitality, or parthenogenesis, has long been called upon to explain malaria relapse. The process, as described by Schaudinn, is too familiar to need repetition here. Despite the objections which have been raised in recent years against this interpretation, it must be admitted that no *à priori* difficulty stands in its way. It is evident from experiments that the protoplasm of an old race is more stabile than that of a young race, possibly due to accumulation of products of metabolism in the former, either for a useful purpose, as in the storage of yolk material in a female cell, or for some harmful purpose, as in

Paramecium caudatum during depression. In either case if a labile protoplasm can be restored resulting in chemical activities which ultimately bring about dissolution of these formed products, then renewed vitality is the outcome. Asexual reorganization effects this result, but the same result was produced artificially by the use of salts in my experiments with *Paramecium caudatum* during conditions of depression, and in cases where the cell body was visibly loaded with products which it could not automatically dispose of. The splendid results which Bass has obtained in cultivating *Plasmodium* in vitro and in the presence of sugar, indicate the possibility of malaria organisms while in a stabile condition being similarly changed into a labile condition by changes in the blood content of the host. Changes thus set up might well be the equivalent of asexual reorganization or parthenogenesis, or the equivalent of fertilization in restoring vitality.

In this sketch of the protozoan life cycle I have endeavored to give a comprehensive though somewhat speculative account of the different phases of vitality which may apply equally well to any type of Protozoa. Cell division, reorganizing encystment or its equivalent, and conjugation, are all regarded as phenomena of the same general character but differing in degree, the effect in each step being the restoration of the protoplasm to a condition more or less free from cumulative metabolic differentiations.

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